

WHAT IS CLAIMED IS:

global

1. A recombinant retroviral vector, said vector comprising in operable combination, a 5' LTR and a 3' LTR derived from a retrovirus of interest, and an insertion site for a gene of interest, wherein said vector does not contain a complete gag, env, or pol gene.

2. A recombinant retroviral vector according to Claim 1, said vector further comprising, a portion of a gag coding sequence adjacent to said Psi packaging site

sub a2

3. A recombinant retroviral vector according to Claim 2, wherein said gag coding sequence comprises, a splice donor site and a splice acceptor site, wherein said splice acceptor site is located upstream from said gene of interest.

4. A recombinant retroviral vector according to Claim 3, said vector further comprising gag transcriptional promoter functionally positioned such that a transcript of a nucleotide sequence inserted into said insertion site is produced, wherein said transcript comprises gag 5' untranslated region.

5. A recombinant retroviral vector according to Claim 4, wherein said vector does not contain a selectable marker.

sub a3

5. A recombinant retroviral vector according to Claim 5, wherein said vector is MFG having the identifying characteristics of ATCC 68,754.

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6 1. A recombinant retroviral vector according to Claim 1, said vector further comprising a gene for expression inserted into said insertion site.

a 5 7 8. A recombinant retroviral vector according to Claim 7, wherein said gene for expression is selected from the group consisting of: a hormone, an enzyme, ~~and a receptor~~, ~~and a drug~~.

a 10 8 8.1 A recombinant retroviral vector according to Claim 8, wherein said gene for expression ~~is~~ <sup>encodes</sup> factor VIII or tPA.

that 15 10. A recombinant retroviral vector according to Claim 1, said vector further comprising, an alpha globin transcriptional promoter.

a 20 10 11. A recombinant retroviral vector according to Claim 10, said vector further comprising, a portion of the 5' untranslated region of the ~~alpha~~ <sup>alpha</sup>-globin gene that is naturally joined to said alpha-globin transcriptional promoter.

25 11 12 10. A recombinant retroviral vector according to Claim 11, said vector further comprising, an enhancer sequence, wherein said enhancer is not in said 5' or 3' LTR.

30 12 13 11. A recombinant retroviral vector according to Claim 12, wherein an enhancer sequence is located upstream from said transcriptional promoter.

35 13 14 12. A recombinant retroviral vector according to Claim 13, wherein said enhancer sequence is a cytomegalovirus enhancer sequence.

Sub 85

15. A recombinant retroviral vector according to Claim 14, wherein said vector is  $\alpha$ -SGC and having the identifying characteristics of ATCC No. 68755.

5 <sup>15</sup> 16. A recombinant retroviral vector according to Claim <sup>15</sup> 15, wherein said 3' LTR does not contain a functional enhancer sequence.

10 <sup>16</sup> 17. A recombinant retroviral vector according to Claim <sup>16</sup> 16, said vector further comprising, a gene for expression inserted into said insertion site.

a <sup>17</sup> 18. A recombinant retroviral vector according to Claim <sup>17</sup> 17, wherein said gene for expression is selected from the group consisting of a hormone, an enzyme, ~~and a receptor~~, ~~and a drug~~.

a <sup>18</sup> 19. A recombinant retroviral vector according to Claim <sup>18</sup> 18, wherein said gene for expression <sup>encodes</sup> is factor VIII or tPA.

a <sup>19</sup> 20. A recombinant retroviral cell line wherein said cell line has been transfected with the coding sequence of a retroviral vector of any <sup>one</sup> of Claims 1 to 19.

Sub 86 21. A recombinant retroviral vector comprising in operable combination, a 5' LTR and a 3' LTR derived from a murine leukemia virus, and an insertion site for a gene of interest, wherein said vector does not contain a complete gag, env, or pol gene.

20 <sup>21</sup> 22. The recombinant retroviral vector of Claim 21, further comprising an exogenous enhancer.

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<sup>22</sup>  
~~23~~. The recombinant retroviral of Claim <sup>21</sup>/~~22~~,  
wherein the exogenous enhancer is derived from a  
myeloproliferative sarcoma virus.

<sup>23</sup>  
5 <sup>21</sup>/~~22~~. The recombinant retroviral vector of Claim  
<sup>22</sup>/~~23~~ wherein the exogenous enhancer is derived from  
~~Moloney~~ Friend Virus.

<sup>24</sup>  
10 <sup>20</sup>/~~21~~, <sup>22</sup>/~~22~~, <sup>23</sup>/~~23~~, or <sup>24</sup>/~~24~~, further comprising a B2 mutation.

<sup>25</sup>  
15 <sup>20</sup>/~~21~~, <sup>22</sup>/~~22~~, <sup>23</sup>/~~23~~, or <sup>24</sup>/~~24~~, wherein the 3' LTR is replaced with  
a 3' LTR derived from a myeloproliferative sarcoma  
virus.

<sup>26</sup>  
<sup>25</sup>/~~26~~. The recombinant retroviral vector of Claim  
<sup>26</sup>/~~26~~, further comprising a B2 mutation.

<sup>27</sup>  
20 <sup>20</sup>/~~21~~, <sup>22</sup>/~~22~~, <sup>23</sup>/~~23~~, or <sup>24</sup>/~~24~~, wherein the 5' LTR is replaced with  
a 5' LTR derived from a myeloproliferative sarcoma  
virus.

<sup>28</sup>  
25 <sup>27</sup>/~~28~~. The recombinant retroviral vector of Claim  
<sup>28</sup>/~~28~~, further comprising a B2 mutation.

<sup>29</sup>  
30 <sup>20</sup>/~~21~~, <sup>22</sup>/~~22~~, <sup>23</sup>/~~23~~, or <sup>24</sup>/~~24~~, wherein both the 5' LTR and the 3'  
LTR are respectively replaced with a 5' LTR and a 3'  
LTR derived from a myeloproliferative sarcoma virus.

<sup>30</sup>  
35 <sup>29</sup>/~~30~~. The recombinant retroviral vector of Claim  
<sup>30</sup>/~~30~~, further comprising a B2 mutation.

32. A method for genetically modifying a hematopoietic stem cell such that the cell expresses the product of a gene of interest, comprising:

- 5 (a) engineering a recombinant retroviral vector according to Claim 21 to contain the gene of interest at the insertion site; and
- (b) transducing the hematopoietic stem cell with the recombinant retroviral vector.

10 33. A method of treating a hematologic disease characterized by a defective gene in a hematopoietic cell in a patient comprising:

- (a) isolating allogenic, HLA-identical bone marrow cells from a donor;
- 15 (b) transducing the donor bone marrow cells with a recombinant retroviral vector, according to Claim 21, engineered to contain a normal gene corresponding to the defective gene at the vector insertion site;
- 20 (c) culturing the transduced donor bone marrow cells;
- (d) destroying the patient's immune system; and,
- (e) administering approximately  $2-6 \times 10^8$
- 25 transfused donor bone marrow cells per kilogram body weight to the patient via intravenous infusion following destruction of the patients immune system.

30 34. An improved synthetic vascular graft, the improvement comprising a lining of autologous endothelial cells genetically modified to produce human tissue-type plasminogen activator on the luminal surface of the graft, wherein said endothelial cells

35 have been modified prior to implantation of the graft

by transducing parental endothelial cells with a recombinant retroviral vector, according to any of Claims 1-19, engineered to contain the coding sequence for human tissue-type plasminogen activator at the  
5 vector insertion site.

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Add C2

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G5

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